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TI Pyrophosphorolysis by Type II DNA polymerases: implications for
pyrophosphorolysis-activated polymerization.
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AB We find that Type II DNA polymerases can catalyze pyrophosphorolysis, the
reverse reaction of DNA polymerization. This property is applied
utilizing pyrophosphorolysis-activated polymerization (PAP), a method of
nucleic acid amplification using serial coupling of pyrophosphorolysis and
polymerization. PAP can be used for ultrarare allele detection (detection
of minimal residual disease and cancer risk assessment through measurement
of mutation load) and for microarray-based scanning for unknown mutations.
Herein, we show that Type II DNA polymerases efficiently catalyze
template-dependent pyrophosphorolysis to activate oligonucleotides blocked
at their 3' termini with acyclonucleotides in which a
2-hydroxyethoxymethyl group substitutes for the 2'-deoxyribofuranosyl
sugar. Type II archeon DNA polymerases Vent (exo-) and Pfu
(exo-) can be utilized for PAP or a bidirectional form of PAP with
acyclonucleotide-blocked oligonucleotides, but not with
dideoxynucleotide-blocked oligonucleotides. In contrast, a Type I DNA
polymerase, TaqFS, can utilize either acyclonucleotide-blocked or
dideoxynucleotide-blocked oligonucleotides. These findings expand the
potential of nascent PAP technology.